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Interaction of bovine serum albumin (BSA) with ionic surfactants evaluated by electron paramagnetic resonance (EPR) spectroscopy

Diógenes de Sousa Neto[a,b], Carlos Ernesto Garrido Salmon[c,1], Antonio Alonso[d], Marcel Tabak[a,*]

[a] Instituto de Química de São Carlos, Universidade de São Paulo, Cx. Postal 780, CEP 13560-970, São Carlos, SP, Brazil
[b] Instituto de Física de São Carlos, Universidade de São Paulo, Cx. Postal 66318, CEP 05315-970, São Carlos, SP, Brazil
[c] Departamento de Física, Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, CEP 49100-000, São Cristóvão, SE, Brazil
[d] Instituto de Física, Universidade Federal de Goiás, Goiânia 74001-970, GO, Brazil

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ABSTRACT

EPR spectra of 5- and 16-doxyl stearic acid nitroxide probes (5-DSA and 16-DSA, respectively) bound to bovine serum albumin (BSA) revealed that in the presence of ionic surfactants, at least, two label populations coexist in equilibrium. The rotational correlation times (τ) indicated that component 1 displays a more restricted mobility state, associated to the spin labels bound to the protein; the less immobilized component 2 is due to label localization in the surfactant aggregates. For both probes, the increase of surfactant concentration leads to higher motional levels of component 1 followed by a simultaneous decrease of this fraction of nitroxides and its conversion into component 2. For 10 mM cethyltrimethylammonium chloride (CTAC), the nitroxides are 100% bound to the protein, whereas at 10 mM N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (HPS) and sodium dodecyl sulfate (SDS) the fractions of bound nitroxides are reduced to 18% and 86%, respectively. No significant polarity changes were observed in the whole surfactant concentration range for component 1. Moreover, at higher surfactant concentration, component 2 exhibited a similar polarity as in the pure surfactant micelles. For 16-DSA the surfactant effect is different: at 10 mM of HPS and CTAC the fractions of bound nitroxides are 76% and 49%, respectively, while at 10 mM SDS they are present exclusively in a micellar environment, consistent with 100% of component 2. Overall, both SDS and HPS are able to effectively displace the nitroxide probes from the protein binding sites, while CTAC seems to affect the nitroxide binding to a significantly smaller extent.

1. Introduction

Serum albumin is an abundant plasma protein that plays an important role in transport of many small molecular weight compounds in blood plasma [1]. Its remarkable ability for binding fatty acids [1] has motivated our group to use spin-labeled derivatives of stearic acids to monitor conformational changes around its binding sites in response to addition of ionic surfactants [2,3]. Fig. 1 shows the structure of spin labels and surfactants used in this work. Despite numerous reports involving the binding of fatty acids and surfactants to serum albumin [4–10], a complete understanding of these interactions is not yet well established.

Recent crystallographic studies [11–14] of the human serum albumin (HSA) complexed with long-chain fatty acids (C16:0 and C18:0) have revealed the existence of seven binding sites. Besides that, for medium-chain fatty acids (C10:0 to C14:0), the data indicated the presence of further sites, yielding a total of 11 different binding sites [13]. Although these findings are concerning HSA, some of these binding properties could be extended to bovine serum albumin (BSA) due to their high sequential identity, estimated to be around 76% [1].

The first crystallographic analyses of HSA [15,16] revealed that it is constituted by three homologous α-helical domains (I–III), and each domain contains 10 helices, divided into a six-helix and a four-helix sub-domains (named A and B). Moreover, the first four helices of A and B form similar anti-parallel α-helix bundles. Fig. 2 represents the crystal structure of HSA with the stearic acid (C18:0) molecules bound to the protein, where the numbers 1–7 represent the localization of the reported binding sites of different affinities [13,17]. The sites 1–5 have important common features: in each case, the hydrocarbon tail of the fatty acid is accommodated inside a deep hydrophobic cavity (pocket), while the carboxyl moiety is bound to two or three basic or polar aminoacid residues by electrostatic/polar interactions. These crystallographic studies have evidentiated the specific protein residues involved in salt bridges or hydrogen bonds for sites 1–5. Differently from sites 1–5, sites 6 and 7 do not display a clear evidence of polar interactions

* Corresponding author. Fax: +55 16 33739982.
E-mail address: marcel@sc.usp.br (M. Tabak).
[1] Present address: Departamento de Física e Matemática, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14040-901 Ribeirão Preto, SP, Brazil.

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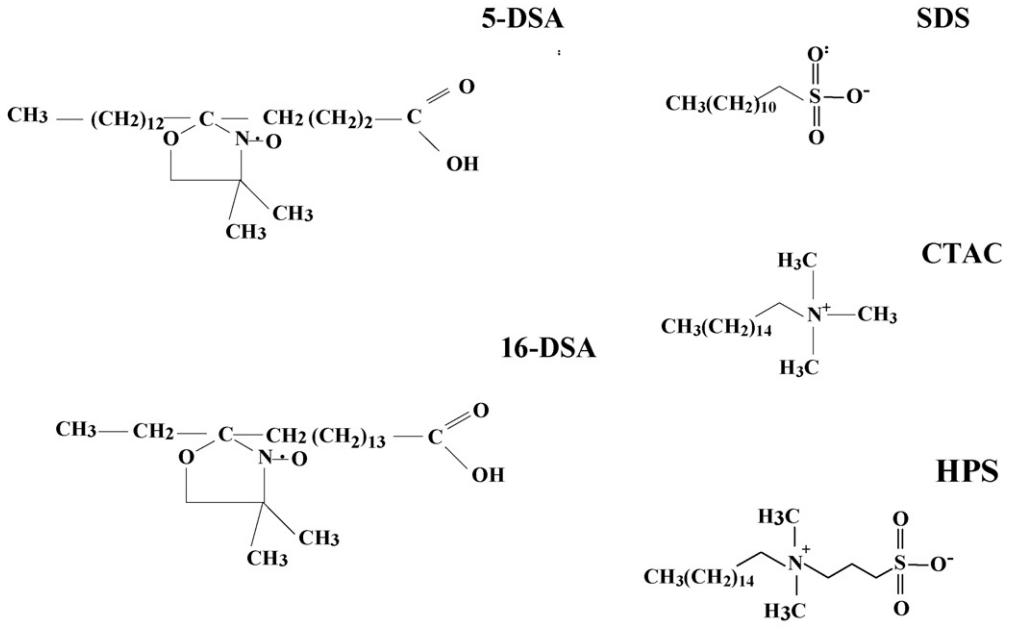

Fig. 1. Chemical structures of nitroxides and surfactants used in this work. The counter ions Na^+ for SDS and Cl^- for CTAC are omitted.

that keep in place the carboxylate head of the fatty acid, suggesting they are lower affinity fatty acid binding sites. Crystallographic studies are not able to address the question regarding the determination of binding affinities for these sites since saturation concentration of the ligands is always used in the crystallization experiments.

Recent NMR studies [18,19] involving the titration of HSA with [^{13}C]carboxyl-labeled long-chain fatty acid have demonstrated the existence of three NMR chemical shifts that are associated with the high-affinity binding sites on the protein. Based on studies using HSA mutants, sites 4 and 5 were interpreted as being two of the three high-affinity long-chain fatty acid binding sites on HSA. These new investigations have provided further insights concerning the localization and relative affinities of different ligands for the HSA binding sites. It is worth of notice that binding of ^{13}C-enriched fatty acids to HSA reproduced older experiments using BSA [20,21], confirming the high homology of the two proteins and probable similarity of their fatty acid binding sites.

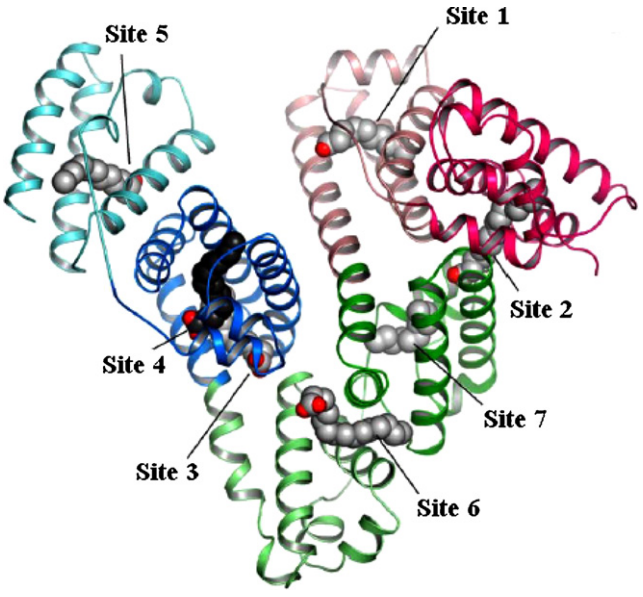

Fig. 2. Crystal structure of HSA indicating the location of the seven binding sites for the stearic acid (1–7). The figure was obtained and modified from Ref. [19].

Our present work proposes to evaluate the dynamics of spin-labeled derivatives of stearic acids (Fig. 1) distributed in the binding sites of BSA, in the absence and in the presence of different ionic surfactants. Studies of BSA–surfactant interactions have been performed for many years aiming to understand how surfactant binding affects the protein structure and function [4–10,22]. Most of these studies were performed with anionic sodium dodecyl sulfate (SDS), although some of them used also cationic CTAB. In the present report, we have investigated some properties involved in the interaction of BSA with anionic sodium dodecyl sulfate, zwitterionic N-hexadecyl-N,N-dimethyl-3-ammonium-1-propane sulfonate (HPS), and cationic cethyltrimethylammonium chloride (CTAC) surfactants (Fig. 1). It is well known that the affinity of the protein for ionic ligands bearing alkyl side chain depends on the hydrophobic character of the chain and the sign of the charged group [22,23]. Molecules with long-chain fatty acids and negative ionic groups, such as stearic acids, are more tightly bound to the protein sites as compared to the molecules containing shorter side chains or positively charged ionic groups [1,22,23]. In fact, we have reported in previous work that at stoichiometric concentrations, the order of surfactant binding constants to BSA is SDS > HPS > CTAC [4,24].

Electron paramagnetic resonance (EPR) spectra of spin labels bound to BSA have been analyzed previously using the non-linear least-squares (NLSL) simulation program [25–27]. The results obtained for doxyl stearates labeled at different positions in the methylene chain showed an anisotropy in the rotational diffusion when incorporated to BSA [27]. Based on NMR results, these findings were interpreted assuming that the hydrocarbon chain of the spin labels are inserted into a protein channel, with the formation of a double-hydrogen bond between the negatively charged carboxylate group of the spin label and the positively charged guanidine group of Arg-335 residue, located in the domain III of BSA [27]. This site seems to exhibit some similar characteristics with the fatty acid site 5 of the HSA described in the crystal structure of the complex [11,17], where the tip of the tail of stearic acid projects through the far side of the channel and its carboxylate headgroup also interacts